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Final Report Summary
J. Robert Bridges - Alexandria, La.

A SURVEY OF THE MYCANGIAL FUNGI IN ENDEMIC AND EPIDEMIC SOUTHERN PINE BEETLE POPULATIONS

Females from eight endemic populations of the southern pine beetle, *Dendroctonus frontalis* Zimm., were examined to determine which of two fungi were present in the mycangium. The fungi, *Ceratocystis minor* (Hedgcock) Hunt var. *barrasii* Taylor, and an unidentified basidiomycete were found together in 10 to 20% of the beetles. Less than 10% of each population had neither fungus in the mycangium while 13 to 45% had *C. minor* var. *barrasii* alone. The basidiomycete was the most prevalent fungus in most populations and occurred alone in 38 to 75% of the beetles. Frequency of occurrence of the fungi varied significantly among the eight populations studied. The mean mass of individual females varied significantly ($P < 0.01$) depending on which fungus was present in the mycangium. Weights varied as follows: females with only the basidiomycete > those with both fungi > those with only *C. minor* var. *barrasii* > those with neither fungus. Brood increase ratio and brood survival were significantly related to the frequency of occurrence of the mycangial fungi. The strongest relationship was found between these indices of population trends and the frequency of occurrence of the basidiomycete. Both indices were negatively correlated with the percentage of *C. minor* var. *barrasii* in a population.

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A SURVEY OF THE MYCANGIAL FUNGI IN ENDEMIC AND EPIDEMIC SOUTHERN PINE BEETLE POPULATIONS

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FINAL REPORT

A SURVEY OF THE MYCANGIAL FUNGI IN ENDEMIC AND EPIDEMIC SOUTHERN PINE BEETLE POPULATIONS

by

J. Robert Bridges

The objectives of this study were to determine the frequency of occurrence of mycangial fungi among a) endemic and epidemic southern pine beetle (SPB) populations, b) SPB populations from various geographic locations, and c) SPB collected at various seasons of the year. This study was planned with the belief that knowledge of the occurrence of mycangial fungi in SPB populations would provide valuable insights into the roles of these fungi in beetle biology.

The results of this study were significant because they demonstrated that beetle vigor and survival depend, at least in part, on which fungus the beetles carry. But, the results also raised many other research questions that are outside the scope of this study. For example, the large variations found in the fungi carried by beetles from different trees makes the interpretations of differences among geographic locations difficult. Although mycangial fungi were shown to be related to beetle weight, brood survival increase, and brood increase ratio, these indices are not easily translated into predictors of beetle population buildup or collapse. Variations in the frequency of occurrence of fungi among beetles from tree to tree could be related to certain tree or stand characteristics. This points out the need for more information on host tree/beetle interactions.

This study provided important information concerning the possible role of mycangial fungi in SPB population dynamics. The results also brought up other research problems that could not be adequately answered by this study. This study will be replaced with another study which will be broader in scope. The following manuscript, which has been accepted for publication in Environmental Entomology, will serve as the final report.

This study is closed.

Introduction

The southern pine beetle, *Dendroctonus frontalis* Zimm. (SPB), lives in association with two symbiotic fungi that are propagated and transported in the adult female's prothoracic mycangium (Barras and Perry 1972). These fungi are obligate symbionts, and their absence results in poor development of the beetle (Barras 1973). One of these fungi is *Ceratocystis minor* (Hedgcock) Hunt var. *barrasii* Taylor (Barras and Taylor 1973). Although it is a variety of the bluestain fungus, *C. minor*, it does not stain the phloem or wood. Spores of the bluestain fungus are not carried in the mycangium but are carried on the beetle's exoskeleton or by phoretic mites (Bridges and Moser in press). The other fungus carried in the mycangium is a nonstaining unidentified basidiomycete. The two fungi are seldom intermixed in the mycangium and can be distinguished from one another by microscopic examination of the mycangium (Barras and Perry 1972). Either a single species occupies the whole mycangium, or the two species occur on opposite sides of the mycangium (Barras and Taylor 1973). In a previous study most of the beetles examined carried only the basidiomycete in the mycangium (Barras and Perry 1972).

Because individual beetles usually carry only one fungus in the mycangium, various populations of beetles might have differing frequencies of occurrence of these two fungi. The frequency of occurrence of the mycangial fungi could be related to the success or failure of a beetle population. To investigate these possibilities, beetles from eight locations were examined to determine the frequency of occurrence of the two mycangial fungi in each SPB population. The purpose of the study was to assess whether a predominance of either fungus in a population might be related to beetle mass, brood survival, and brood increase ratio.

Materials and Methods

From August to December 1981, 14 trees were sampled from eight separate infestations (spots) in the Holly Springs and Homochitto National Forests in Mississippi. The number of trees actively infested with southern pine beetle (SPB) in each spot ranged from 1 to about 25. Two of the trees were shortleaf pines (*Pinus echinata* Mill.); the rest were loblolly (*Pinus taeda* L.).

Two trees were sampled at each location except in two cases in which only one tree/spot was available for sampling. Trees were felled for sampling when the broods were primarily pupae or adults. Sample bolts approximately 45 cm long were removed from each tree at 2 and 5 m heights, giving two samples per tree. The bolts were brought to the laboratory and placed in rearing cans. When possible, 100 brood adult females were collected as they emerged from each sample bolt. Four of the samples had less than 100 females/sample. Each beetle was weighed on a Cahn Electrobalance and then examined microscopically to determine which fungus was present in the mycangium. After the brood had emerged, four 93-cm^2 bark samples were removed from four equally-spaced locations around each bolt. Bark samples were examined to determine length of the egg gallery, number of attacks, and number of brood that emerged. Ratio of increase was determined for each sample as number of brood/(2 x number of attacks) (Thatcher and Pickard 1964, Linit and Stephen 1978). Brood survival was determined as brood beetles emerging per egg. The number of eggs was estimated from the linear relationship between oviposition and gallery length (1.59 eggs/cm gallery) reported by Foltz et al. (1976).

The prothorax was removed from each female, and excess tissue was removed from around the mycangium. The mycangium was divided dorsoventrally into two pieces, and each side was mounted on a microscope slide in lactophenol-cotton blue[2/]. The mycangium pieces were examined under 20-40X phase microscopy to determine the presence of the two mycangial fungi (Barras and Perry 1972). Each side of the mycangium was examined separately to determine whether the two fungi were intermixed or whether they occurred on opposite sides of the mycangium.

Based on which fungus was present in the mycangium, each beetle was assigned to one of the following categories: 1) only the basidiomycete present, 2) only C. minor var. barrasii present, 3) both fungi present and 4) neither fungus present. Using the Statistical Analysis System[3/] an analysis of variance (ANOVA) was run to evaluate differences in the mass of beetles among each of the categories. A randomized block design was used with infestation location being the blocking factor. An ANOVA was also performed on the percentages of beetles in each of the four categories to detect differences due to infestation location and sample height on the infested bole. Regression analyses were run to determine the relationship of frequency of occurrence of the mycangial fungi to estimators of SPB population trends (Gagne et al. 1981). Ratio of increase or within-sample bolt survival was the dependent variable; percentage of beetles in one of the four categories was the independent variable.

Results

The frequency of occurrence of mycangial fungi varied significantly among the various SPB populations (infestations) (Table 1). But there were no significant differences in the frequency of occurrence of the fungi between the two sample heights within a tree.

With only one exception, the basidiomycete was the most prevalent fungus in beetles from all infestations. The percentage of beetles having the basidiomycete as the only fungus in the mycangium ranged from 38.5 to 75.5% (Table 1). Beetles having only C. minor var. barrasii comprised the next most prevalent group. The proportion of beetles having both fungi in the mycangium ranged from 10.1 to 20.5% (Table 1). In 97.7% of the beetles that carried both fungi, the fungi occurred on opposite sides of the mycangium. A small percentage (0.5 to 10%) of each population had neither fungus in the mycangium.

The mass of individual SPB females varied significantly ($P < 0.01$) with the fungus present in the mycangium (Table 2). Beetles having only the basidiomycete were heaviest. Beetles having C. minor var. barrasii alone weighed less than beetles having both fungi but weighed significantly more than beetles with neither fungus (Table 2).

Regression analyses showed significant relationships between population trend estimators and the frequency of occurrence of the mycangial fungi (Fig. 1 and 2). Brood increase ratio and brood survival were significantly correlated with the percentage of beetles in each sample that had only the basidiomycete. These estimators were negatively correlated with the absence of fungi. This was as might be expected, because the absence of mycangial fungi has been shown in laboratory experiments to decrease brood production (Barras 1973). Brood increase ratio and brood survival were also negatively correlated with the percentage of beetles having only *C. minor* var. *barrasii* (Fig. 1 and 2). There was no significant correlation between the two estimators and the percentage of beetles that carried both fungi.

Discussion

Results of this study demonstrate that the mean mass of female SPB varied with presence or absence of fungi in the mycangium. Because the size of bark beetles has been shown to be related to fecundity (Reid 1962, Amman 1972, Clarke et al. 1979), the presence of a particular fungus in the population could affect the beetle's reproductive potential. The two estimators of population trends were also related to the presence or absence of the mycangial fungi. Therefore, the predominance of one fungus in a population could significantly affect the beetle's population dynamics.

A higher frequency of occurrence of the basidiomycete compared to *C. minor* var. *barrasii* appears to increase the chances of success of a SPB population based on the estimators of population trends. The basidiomycete also resulted in heavier females which would presumably have a greater reproductive potential. The presence of *C. minor* var. *barrasii* appears to be more conducive to beetle success than complete absence of fungi. Beetles with *C. minor* var. *barrasii* were heavier than beetles without fungi, and brood survival and ratio of increase were less negatively correlated with its presence.

The relationships of the mycangial fungi to beetle mass, brood survival, and ratio of increase suggest that these fungi may affect beetle nutrition. Although the role of the mycangial fungi in the life cycle of SPB is not known, a contribution to beetle nutrition is usually assumed. Changes in the nitrogen and carbohydrate contents of host tree phloem brought about by the SPB/microorganism complex have been described (Barras and Hodges 1969, Hodges et al. 1968). Localized growth of the fungi on the walls of larval galleries might also supply specific nutrients, concentrate nutrients from phloem near larval galleries, or detoxify compounds in the phloem.

Although the two species of fungi occur consistently with the SPB, they affect the beetle's development to different degrees. Because the basidiomycete appears to have a more positive influence than C. minor var. barrasii on beetle success, the question arises as to why SPB has evolved such a close relationship with C. minor var. barrasii. It is possible that under certain conditions (temperature or other climatic conditions, tree or stand characteristics, etc.) C. minor var. barrasii would be as important to SPB success as the basidiomycete. This study examined endemic SPB populations. A similar study during epidemic conditions might discover other relationships between the SPB and its mycangial fungi.

The fungi may also be tree pathogens enabling the beetle to overcome otherwise resistant trees. Although the bluestain fungus associated with SPB has been shown to be pathogenic to pines, its role in killing bark beetle-attacked trees is not clear (Mathre 1964, Whitney and Cobb 1972). Mycangial fungi of other bark beetles, including non-staining fungi, have been implicated in the death of bark beetle-killed trees (Reid et al. 1967, Whitney and Cobb 1972). It is very possible that the fungi have different roles. For example, one fungus might be more important to enable beetles to overcome resistant trees. Definition of the roles of each fungus awaits further experimentation.

The results of this study demonstrate that the frequency of occurrence of the two mycangial fungi varies among SPB populations. The reasons for this variation are not known. Temperature, season, or tree/stand characteristics may influence which fungus predominates in a beetle population. The frequency of occurrence of these fungi relates to population trends of SPB, which could be an important factor regulating SPB outbreaks. Although many factors are probably involved in triggering an outbreak, the occurrence of the symbiotic fungi should be an important consideration in future population dynamics research.

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Footnotes

1/ Received for publication 25 August 1982.

2/ Saturated solution of phenol in lactic acid with 0.05% cotton blue.

3/ SAS Institute, Inc., P. O. Box 1006, Raleigh, NC 27605.

Table 1. Mean frequency of occurrence of mycangial fungi in SPB from eight infestations in Mississippi.

Infestation No.	n[2/]	Percent of population having[1/]			
		Only the basidiomycete	Only *C. minor* var. *barrasii*	Both fungi	Neither fungus
1	370	38.5	47.0	10.9	3.6
2	400	52.0	27.8	13.2	7.0
3	331	49.7	30.2	10.1	10.0
4	400	68.0	14.0	17.0	1.0
5	400	54.5	26.8	16.8	2.0
6	200	63.0	20.0	15.0	2.0
7	200	75.5	13.0	11.0	0.5
8	400	53.8	24.8	20.5	1.0
Mean	338	56.9	25.4	14.3	3.4
		$F = 2.81$	$F = 4.00$	$F = 2.56$	$F = 3.75$
		$P < 0.05$	$P < 0.05$	$P < 0.05$	$P < 0.05$

[1/] Variations among infestations were analyzed by ANOVA using a random model.

[2/] n = Number of beetles examined.

Table 2. Effect of presence or absence of mycangial fungi on beetle mass.

Fungi in mycangium	n[1/]	Mean mass (mg)[2/]
Basidiomycete alone	1497	2.16 a
Both fungi	396	1.98 b
C. minor var. *barrasii* alone	714	1.72 c
Neither fungus	94	1.39 d

1/ n = Total number of beetles examined.

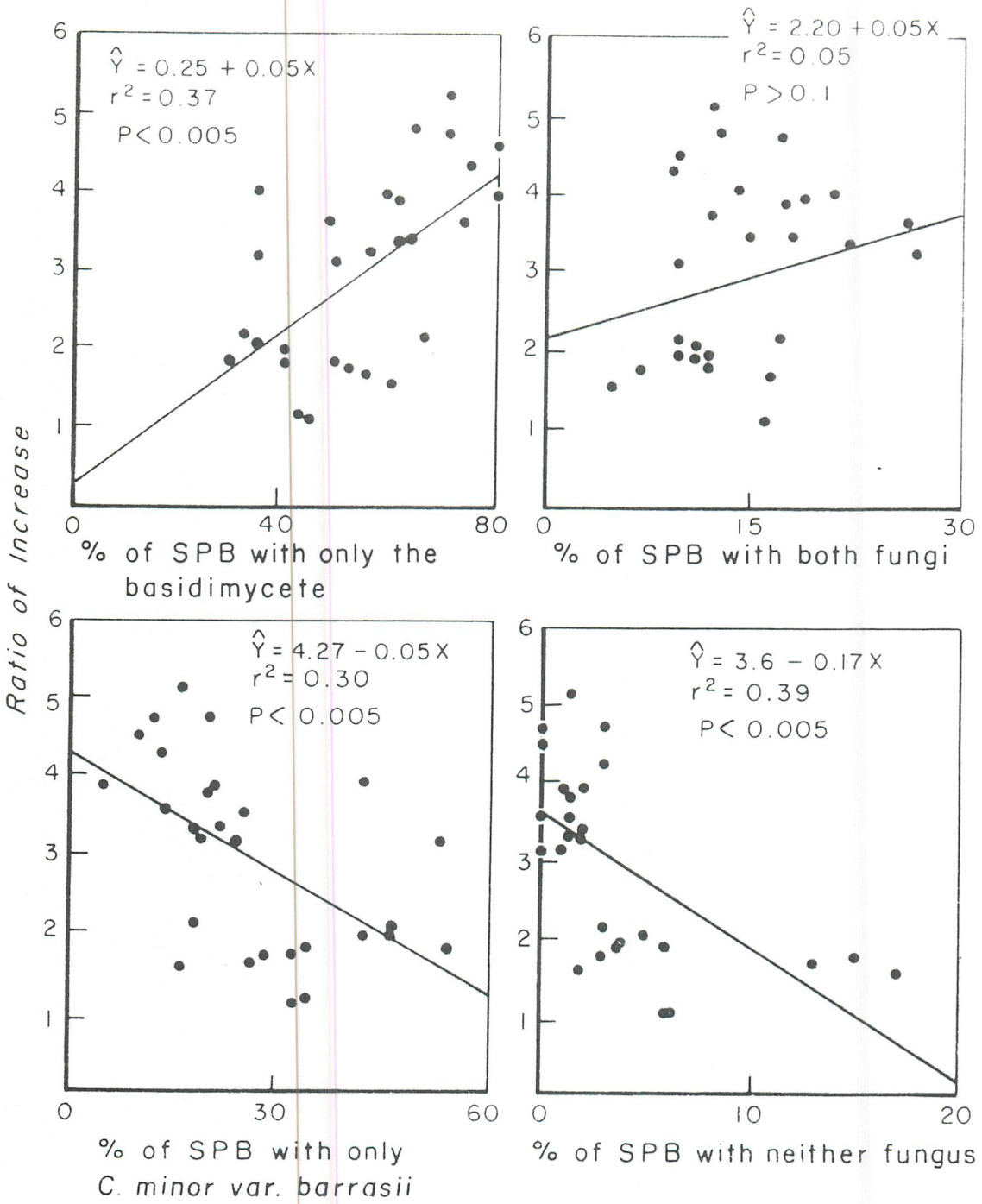
2/ Means not followed by the same letter are significantly different (P =0.01) according to Duncan's multiple range test.

Figures

Fig. 1.--Regressions of ratio of increase against percentage of SPB with fungi in the mycangium.

Fig. 2.--Regressions of brood survival against percentage of SPB with fungi in the mycangium.

Figure 1.


Ratio of Increase
Ŷ = 0.25 + 0.05X
r² = 0.37
P < 0.005
% of SPB with only the basidimycete
Ŷ = 2.20 + 0.05X
r² = 0.05
P > 0.1
% of SPB with both fungi
Ŷ = 4.27 − 0.05X
r² = 0.30
P < 0.005
% of SPB with only C. minor var. barrasii
Ŷ = 3.6 − 0.17X
r² = 0.39
P < 0.005
% of SPB with neither fungus

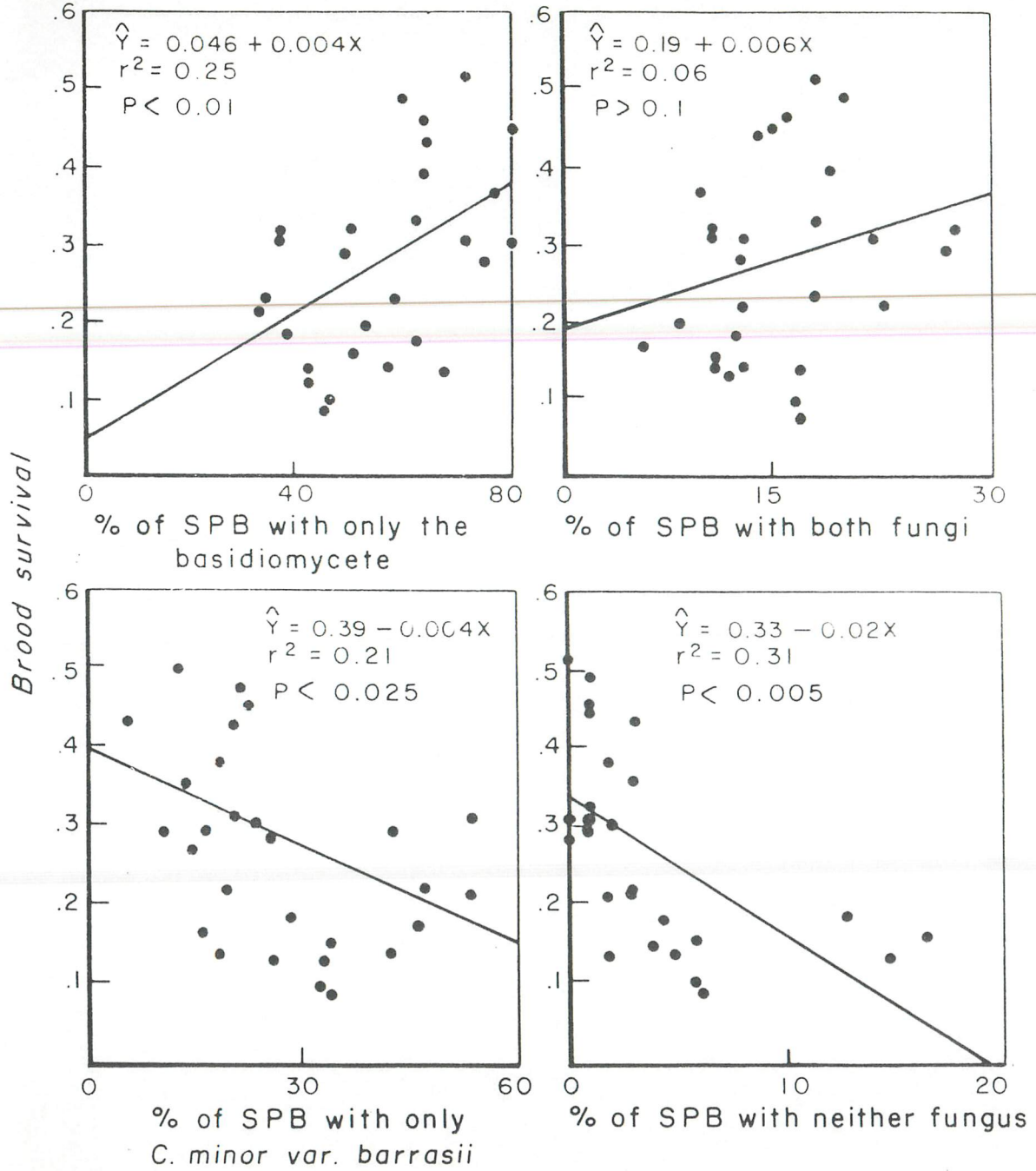

Brood survival
Ŷ = 0.046 + 0.004X
r² = 0.25
P < 0.01
% of SPB with only the basidiomycete
Ŷ = 0.19 + 0.006X
r² = 0.06
P > 0.1
% of SPB with both fungi
Ŷ = 0.39 − 0.004X
r² = 0.21
P < 0.025
% of SPB with only C. minor var. barrasii
Ŷ = 0.33 − 0.02X
r² = 0.31
P < 0.005
% of SPB with neither fungus

Figure 2.

UNITED STATES DEPARTMENT OF AGRICULTURE
FOREST SERVICE

SO-AL

REPLY TO: 4050 Research Programs

January 20, 1983
(RWU 2203)

SUBJECT: Final Report for FS-SO-2203-1.46, "A survey of the mycangial fungi in endemic and epidemic southern pine beetle populations," by J. Robert Bridges

TO:

TO: S. J. Barras, AD-W

A copy of subject final report is attached for your information. Also attached is the final report summary. The manuscript, accepted for publication by Environmental Entomology, and entitled "Mycangial fungi of Dendroctonus frontalis (Coleoptera: Scolytidae) and their relationship to beetle population trends", comprises the final report.

Bettie J. Mallard

for PETER L. LORIO, JR.
Project Leader
Forest Insect Research

Enclosures

PLLorio,Jr./bjm

Mycangial Fungi of *Dendroctonus frontalis* (Coleoptera:Scolytidae) and their Relationship to Beetle Population Trends

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Abstract

Females from eight endemic populations of the southern pine beetle, Dendroctonus frontalis Zimm., were examined to determine which of two fungi were present in the mycangium. The fungi, Ceratocystis minor (Hedgcock) Hunt var. barrasii Taylor, and an unidentified basidiomycete were found together in 10 to 20% of the beetles. Less than 10% of each population had neither fungus in the mycangium while 13 to 47% had C. minor var. barrasii alone. The basidiomycete was the most prevalent fungus in most populations and occurred alone in 38 to 75% of the beetles. Frequency of occurrence of the fungi varied significantly among the eight populations studied. The mean mass of individual females varied significantly ($P < 0.01$) depending on which fungus was present in the mycangium. Weights varied as follows: females with only the basidiomycete > those with both fungi > those with only C. minor var. barrasii > those with neither fungus. Brood increase ratio and brood survival were significantly related to the frequency of occurrence of the mycangial fungi. The strongest relationship was found between these indices of population trends and the frequency of occurrence of the basidiomycete. Both indices were negatively correlated with the percentage of C. minor var. barrasii in a population.

Running head: Bridges: Mycangial fungi of Dendroctonus frontalis